(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 October 2004 (14.10.2004)

PCT

(10) International Publication Number
WO 2004/087758 A2

(51) International Patent Classification[7]: C07K 16/00

(21) International Application Number: PCT/US2004/009354

(22) International Filing Date: 26 March 2004 (26.03.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/457,898 26 March 2003 (26.03.2003) US

(71) Applicant *(for all designated States except US)*: **NEOPHARM, INC.** [US/US]; 150 Field Drive, Suite 195, Lake Forest, IL 60045 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GATELY, Stephen, T.** [CA/US]; 357 E. Shady Pines Court, Palatine, IL 60067 (US). **WANASKI, Stephen, P.** [US/US]; 1301 West Fletcher, suite 503, Chicago, IL 60657 (US).

(74) Agents: **HEFNER, M., Daniel** et al.; Leydig, Voit & Mayer, Ltd., Two Prudential Plaza, Suite 4900, 180 North Stetson Av., Chicago, IL 60601-6780 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IL 13 RECEPTOR ALPHA 2 ANTIBODY AND METHODS OF USE

(57) Abstract: The invention provides an antiboy direted against an IL13 receptor alpha 2 (IL13-Rα2) and methods of using the antibody to detect an localize IL13-Rα2, to diagnostic a disease characterized by expression of IL13-Rα2, and to kill a cell that expresses an IL13-Rα2.

WO 2004/087758 A2

IL13 RECEPTOR ALPHA 2 ANTIBODY AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to co-pending United States Provisional Patent Application 60/457,898, which was filed on March 26, 2003, and the contents of which are hereby incorporated herein in their entirety by reference.

FIELD OF THE INVENTION

[0002] This invention pertains to an IL13 receptor alpha 2 (IL13-Rα2) antibody and methods of using IL13-Rα2 antibodies.

BACKGROUND OF THE INVENTION

[0003] Malignant glioma, including glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA), occurs in approximately 17,500 patients annually in the United States. Despite an aggressive multimodal approach to its treatment, no curative therapy is known. Median survival expectation is 9-12 months from diagnosis for GBM and 24-48 months for AA. Despite numerous investigational trials, patients with a recurrence of malignant glioma after initial radiotherapy do not live long.

[0004] One approach to eradicating tumor cells is to target cytotoxic agents to the cells. To accomplish this, antibodies or growth factors that bind to cells can be attached to cytotoxic molecules. The binding sites on such cells are known as cell receptors. This method is selective in situations where the targeted receptors are present in substantially higher amounts on target cells than in normal cells. Selectivity is desirable as it minimizes toxicity to normal cells. Exceptionally high levels of the alpha 2 receptor for Interleukin 13 (IL13-Rα2) have been identified in a number of tumor cells, including malignant gliomas. In contrast, only a few types of normal cells express IL13-Rα2 and only at low levels. Consequently, antibodies that bind IL13-Rα2 have the potential to be an effective tool for the diagnosis, screening, and treatment of diseases associated with the expression of IL13-Rα2 on cell surfaces.

[0005] In this regard, overexpression of the IL13-Rα2 in a target (i.e., tumor) cell may predict a positive response to a therapeutic agent that targets IL13-Rα2. Moreover, localization of IL13-Rα2 expression to a particular cell or tissue type will allow physicians to more precisely identify those tissues affected by an IL13-Rα2-associated disease. In addition to diagnostic and preventative applications, antibodies or growth factors (i.e., IL13) that bind IL13-Rα2, when combined with a cytotoxic agent, also have the potential to be a highly effective therapeutic agent for the treatment of IL13-Rα2-expressing tumor cells. Despite the potential for such useful applications, currently each is hindered by difficulty in

detecting IL13-Rα2 expression. Thus, there remains a need for compositions and methods that can be used to reliably and efficiently detect and localize IL13-Rα2 expression in cells, particularly tumor cells.

BRIEF SUMMARY OF THE INVENTION

[0006] The invention provides an isolated antibody or antigen-binding fragment thereof directed against an IL13-Rα2 that binds an epitope comprising or consisting essentially of an amino acid sequence of SEQ ID NO:1. The invention also provides a method for detecting or localizing an IL13-Rα2 polypeptide *in vitro* comprising (a) contacting a sample or cell suspected of containing the IL13-Rα2 with an isolated antibody that binds the IL13-Rα2, and (b) detecting binding of the IL13-Rα2 antibody to the IL13-Rα2. Another aspect of the invention provides a method for diagnosing a disease characterized by expression of an IL13-Rα2 comprising contacting a cell with an isolated antibody that binds the IL13-Rα2, wherein the detectable binding of the IL13-Rα2 antibody indicates expression of the IL13-Rα2, and the disease is diagnosed. Another aspect of the invention provides a method for killing a cell that expresses an IL13-Rα2 comprising contacting the cell with an isolated antibody that binds to the IL13-Rα2 and is conjugated to a cytotoxic agent, such that the IL13-Rα2 antibody binds the IL13-Rα2 and the cytotoxic agent contacts the cell, whereby the cell is killed. These and other advantages of the invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

DETAILED DESCRIPTION OF THE INVENTION

[0007] In one aspect, the invention provides an isolated antibody or antigen-binding fragment thereof directed against IL13-Rα2. The full-length sequence of the IL13-Rα2 cDNA and protein are set forth as SEQ ID NOs: 3 and 4, respectively. Any antibody (or fragment thereof) that binds the IL13-Rα2 is suitable for use in the invention. In one embodiment, the antibody or antigen-binding fragment thereof binds an epitope comprising or consisting essentially of an amino acid sequence of SEQ ID NO:1.

[0008] Antibodies, also known in the art as immunoglobulins, are molecules having a specific amino acid sequence, by virtue of which they interact only with the antigen that induced their synthesis in cells of the lymphoid series (especially plasma cells), or with an antigen closely related to it. The term "antigen" refers to any molecule that can bind specifically to an antibody. An antigen that can induce antibody production is typically referred to in the art as an immunogen. Antibodies typically are produced in response to infection or immunization, bind to and neutralize pathogens, or prepare pathogens for uptake and destruction by phagocytes (see, e.g., C.A. Janeway et al. (eds.), *Immunobiology*,

5th Ed., Garland Publishing, New York, NY (2001)). The general structure and function of antibody molecules are well known in the art.

[0009] As used herein, an "isolated" antibody (or fragment thereof) refers to at least one antibody molecule (or fragment thereof) that has been isolated, or is otherwise free of, the bulk of the total antibodies circulating in the bloodstream of an animal. Total isolation from all other antibodies, however, is not necessary. Indeed, the inventive antibody composition can be polyclonal, in some embodiments. In other words, an antibody is "isolated" if it has been changed or removed from its natural *in vivo* environment.

[0010] Methods of generating antibodies using purified polypeptides or synthetic oligonucleotides are known in the art. Generally, such methods typically involve administering a polypeptide antigenic determinant (or an oligonucleotide encoding such an antigenic determinant) mixed with an adjuvant to an organism (e.g., a rabbit, mouse, sheep, etc.), such that antibodies directed against the antigen are produced by the organism (see, e.g., Harlow and Lane (eds.), *Antibodies: A Laboratory Manual*, CSH Press (1988), Salvatore et al., *Biochem. Biophys. Res. Comm.*, *294*, 813-817 (2002), and U.S. Patents 5,776,457 and 5,614,191). Specific antibodies raised against the immunizing antigen can be isolated and purified from animal serum using any suitable method known in the art. Such methods include, for example, affinity chromatography, in which immunized serum is applied to beads loaded in a column that are covalently bound to the antigen of interest. Non-specific antibodies and other serum proteins are washed away, leaving only antigen-specific antibodies bound to the antigen coated beads, which are eluted by adjusting the pH, temperature, or salt concentration of the reaction conditions. Other suitable methods for antibody isolation and purification are disclosed in, for example, Published U.S. Patent Application No. 20020197266/A1, U.S. Patent 5,776,457, and Janeway et al., *supra*.

[0011] While the inventive antibody (and composition comprising the same) preferably comprises an antibody directed against an IL13-Rα2, antibody fragments that recognize and bind one or more antigens of an IL13-Rα2 also are within the scope of the invention. In this respect, proteolytic cleavage of an intact antibody molecule can produce a variety of antibody fragments that retain the ability to recognize and bind antigens. For example, limited digestion of an antibody molecule with the protease papain typically produces three fragments, two of which are identical and are referred to as the Fab fragments, as they retain the antigen binding activity of the parent antibody molecule. Alternatively, cleavage of an antibody molecule with the enzyme pepsin normally produces two antibody fragments, one of which retains both antigen-binding arms of the antibody molecule, and is thus referred to as the $F(ab')_2$ fragment. Alternatively, a single-chain Fv antibody fragment, which consists of a truncated Fab fragment comprising the variable (V) domain of an antibody heavy chain linked to a V domain of a light antibody chain via a synthetic peptide, can be generated

using routine recombinant DNA technology techniques (see, e.g., Janeway et al., *supra*). Antibody fragments of the present invention, however, are not limited to these exemplary types of antibody fragments. Any suitable antibody fragment that recognizes and binds IL13-Rα2 is within the scope of the present invention. Antibody-antigen binding can be assayed using any suitable method known in the art, such as, for example, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), Western blot, immunoprecipitation, and competitive inhibition assays (see, e.g., Janeway et al., *supra,* and Published U.S. Patent Application No. 20020197266/A1).

[0012] Antibodies (or antibody fragments) that bind an IL13-Rα2 produced in accordance with the methods disclosed herein can be polyclonal antibodies (or antibody fragments), or monoclonal antibodies (or antibody fragments). As used herein, "polyclonal" antibodies (or antibody fragments) refer to heterogeneous populations of antibody molecules (or antibody fragments), typically obtained from the sera of immunized animals. "Monoclonal" antibodies (or antibody fragments) refer to homogenous populations of antibody molecules (or antibody fragments) that are specific to a particular antigen. Monoclonal antibodies typically are produced by a single clone of B lymphocytes ("B cells"). Monoclonal antibodies (or antibody fragments) may be obtained using a variety of techniques known to those skilled in the art, including standard hybridoma technology (see, e.g., Kohler and Milstein, *Eur. J. Immunol.*, *5*, 511-519 (1976), U.S. Patents 4,376,110 and 5,614,191, Published U.S. Patent Application No. 20021972666/A1, Harlow and Lane, *supra*, and Janeway et al., *supra*). In brief, the hybridoma method of producing monoclonal antibodies typically involves injecting any suitable animal, typically and preferably a mouse, with an antigen (i.e., an "immunogen"). The animal subsequently is sacrificed and B cells isolated from its spleen are fused with myeloma cells. A hybrid cell (i.e., a "hybridoma") is produced, which proliferates indefinitely *in vitro* and continuously secretes high titers of an antibody with the desired specificity. Any appropriate method known in the art can be used to identify hybridoma cells that produce an antibody with the desired specificity. Such methods include, for example, ELISA, Western blot analysis, and radioimmunoassay. The population of hybridomas is screened to isolate individual clones, each of which secrete a single antibody species to the antigen. Because each hybridoma is a clone derived from fusion with a single B cell, all the antibody molecules it produces are identical in structure, including their antigen binding site and isotype. Monoclonal antibodies (or antibody fragments) also may be generated using other suitable techniques including EBV-hybridoma technology (see, e.g., Haskard and Archer, *J. Immunol. Methods*, *74*(2), 361-67 (1984) and Roder et al., *Methods Enzymol.*, *121*, 140-67 (1986)), or bacteriophage vector expression systems (see, e.g., Huse et al., *Science*, *246*, 1275-81

(1989)). To prepare monoclonal antibody fragments, recombinant methods typically are employed.

[0013] The inventive antibody (or fragment thereof) can be isolated from or produced in any animal that can be immunized against an antigen or antigenic determinant of an IL13-Rα2. In one embodiment, the antibody desirably is isolated from or produced in an avian species, such as a chicken. Not to adhere to any one particular theory, it is believed that, due to the evolutionary distance between avian species and mammals, avian antibodies react with more epitopes on a mammalian antigen, resulting in signal amplification, and exhibit reduced cross-reactivity with mammalian antibodies and proteins, reducing background effects in immunological assays. In another embodiment, preferably, the antibody is isolated from or produced in a mammal, more preferably a mouse, and most preferably a human. Methods for producing an antibody using animals such as chicken or mice are well known to those skilled in the art and are described herein. In particular, methods for producing polyclonal and monoclonal antibodies in avian species are described in, for example, WO 01/88162 and WO 00/29444. With respect to human antibodies, one of ordinary skill in the art will appreciate that polyclonal antibodies can be isolated from the sera of human subjects vaccinated or immunized with antigenic portions of an IL13-Rα2. Alternatively, human antibodies directed against an IL13-Rα2 can be generated by adapting known techniques for producing human antibodies in non-human animals such as mice (see, e.g., U.S. Patents 5,545,806 and 5,569,825, and Published U.S. Patent Application No. 20020197266/A1).

[0014] One of ordinary skill in the art will appreciate that, while being the ideal choice for therapeutic and diagnostic applications in humans, human antibodies, particularly human monoclonal antibodies, typically are more difficult to generate than mouse monoclonal antibodies. Mouse monoclonal antibodies, however, induce a rapid host antibody response when administered to humans, which can reduce the therapeutic or diagnostic potential of the mouse antibody. To circumvent these complications, the inventive antibody (or fragment thereof) preferably exhibits reduced recognition by the human immune system as compared to an analogous non-human antibody. Most preferably, the inventive antibody is not recognized as "foreign" by the human immune system. To this end, phage display can be used to generate the inventive antibody. In this regard, phage libraries encoding antigen-binding variable (V) domains of antibodies can be generated using standard molecular biology and recombinant DNA techniques (see, e.g., Sambrook et al. (eds.), *Molecular Cloning, A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, New York (2001)). Phage encoding a variable region with the desired specificity are selected for specific binding to the desired antigen, and a complete human antibody is reconstituted comprising the selected variable domain. Nucleic acid

sequences encoding the reconstituted antibody are introduced into a suitable cell line, such as a myeloma cell used for hybridoma production, such that human antibodies having the characteristics of monoclonal antibodies are secreted by the cell (see, e.g., Janeway et al., *supra*, Huse et al., *supra*, and U.S. Patent 6,265,150). Alternatively, monoclonal antibodies can be generated from mice that are transgenic for specific human heavy and light chain immunoglobulin genes. Such methods are known in the art and described in, for example U.S. Patents 5,545,806 and 5,569,825, and Janeway et al., *supra*). Most preferably, the inventive antibody is a humanized antibody. As used herein, a "humanized" antibody is one in which the complementarity-determining regions (CDR) of a mouse monoclonal antibody, which form the antigen binding loops of the antibody, are grafted onto the framework of a human antibody molecule. Owing to the similarity of the frameworks of mouse and human antibodies, it is generally accepted in the art that this approach produces a monoclonal antibody that is antigenically identical to a human antibody but binds the same antigen as the mouse monoclonal antibody from which the CDR sequences were derived. Methods for generating humanized antibodies are well known in the art and are described in detail in, for example, Janeway et al., *supra*, and U.S. Patents 5,585,089 and 5,693,761.

[0015] The inventive antibody (or antibody fragment) may be of any immunoglobulin isotype. The term "isotype," as is used in the art, typically describes the class, subclass, light chain type and subtype of an antibody. One of ordinary skill in the art will appreciate that the five major human immunoglobulin isotypes are immunoglobulin M (i.e., IgM), IgD, IgG, IgA, and IgE, which are typically defined by the structure of the constant regions of the antibody heavy chain. The light chain of a human antibody molecule is typically classified in the art as either a lambda (λ) chain or a kappa (κ) chain. IgG antibodies can be subdivided further into four subtypes (i.e., IgG1, IgG2, IgG3, and IgG4), whereas IgA antibodies typically are subdivided into two subtypes (i.e., IgA1 and IgA2). In embodiments where the inventive antibody is a chicken antibody, the antibody is preferably of the IgY isotype, which is the main serum immunoglobulin in chicken. Chicken IgY antibodies also are referred to in the art as chicken IgG antibodies, as they are the functional equivalent of mammalian IgG in birds. Like mammalian IgG antibodies, chicken IgY antibodies consist of two light chains and two heavy chains, and can be enzymatically cleaved into Fab fragments. IgY can be isolated from serum or collected from the yolks of eggs produced by immunized hens (see, e.g., Warr et al., *Immunol. Today*, *16*, 392-98 (1995) and Haak-Frendscho M., *Promega Notes Magazine*, *46*, 11 (1994)).

[0016] The inventive isolated antibody, or antigen-binding fragment thereof, can be directed against the full-length IL13-Rα2 or a fragment thereof. The structure and function of IL13-Rα2 have been characterized and described in, for example, Caput et al., *J. Biol. Chem.*, *271*, 16921-16926 (1996). Most preferably, the inventive antibody binds an epitope

of an IL13-Rα2 comprising an amino acid sequence of SEQ ID NO:1, or consisting essentially of this sequence. An "epitope," also known in the art as an "antigenic determinant," is a site or an amino acid sequence recognized by an antibody or an antigen receptor. The epitope recognized by the inventive antibody can be derived from a naturally occurring IL13-Rα2, or synthetically generated using routine recombinant DNA and protein technology (see, e.g., Sambrook et al., *supra*).

[0017] In preferred embodiments, the inventive antibody can recognize any epitope comprising a variant or homolog of the polypeptide set forth in SEQ ID NO:1. A variant of the polypeptide can include a polypeptide encoded by a nucleic acid sequence comprising one or more mutations (e.g., point mutations, deletions, insertions, etc.) from the nucleic acid sequence encoding a corresponding naturally occurring protein. By "naturally occurring" is meant that the protein can be found in nature and has not been synthetically modified. Where mutations are introduced in the nucleic acid sequence encoding the polypeptide, such mutations desirably will effect a substitution in the encoded protein whereby codons encoding positively-charged residues (H, K, and R) are substituted with codons encoding positively-charged residues, codons encoding negatively-charged residues (D and E) are substituted with codons encoding negatively-charged residues, codons encoding neutral polar residues (C, G, N, Q, S, T, and Y) are substituted with codons encoding neutral polar residues, and codons encoding neutral non-polar residues (A, F, I, L, M, P, V, and W) are substituted with codons encoding neutral non-polar residues. In addition, a homolog of the polypeptide can be any peptide, polypeptide, or portion thereof, that is more than about 70% identical (preferably more than about 80% identical, more preferably more than about 90% identical, and most preferably more than about 95% identical) to the polypeptide at the amino acid level. The degree of amino acid identity can be determined using any method known in the art, such as the BLAST sequence database.

[0018] Using any of the methods described herein, one of ordinary skill in the art will appreciate that an animal can be immunized to produce antibodies specific for a particular antigen or epitope by administering a suitable composition comprising a polypeptide encoding the antigen or epitope to the animal. Alternatively, a gene transfer vector comprising a nucleic acid sequence encoding the antigen or epitope can be generated and administered to an animal using any suitable method known in the art, such that the antigen or epitope is produced within the animal, resulting in an antibody response against the antigen or epitope within the animal. In this regard, the inventive IL13-Rα2 antibody preferably recognizes an epitope that is encoded by a nucleic acid sequence comprising SEQ ID NO:2, or consisting essentially of this sequence. The inventive antibody also can be generated by immunizing an animal with a nucleic acid sequence that encodes an epitope

comprising any variant, homolog, or functional portion of SEQ ID NO:1, as described previously herein.

[0019] An epitope of an IL13-Rα2 can be identified using any suitable method known in the art. In this regard, nucleic acid sequences encoding peptide fragments of full-length IL13-Rα2 can be cloned into recombinant expression vectors using standard molecular biology techniques (see, e.g., Sambrook et al., *supra*). Putative IL13-Rα2 epitopes can be tested for antigenicity against sera containing IL13-Rα2 antibodies (e.g., sera isolated from a patient suffering from malignant glioma) *in vitro*, or by administering an expression vector encoding a putative epitope to an appropriate laboratory animal and assaying for anti-IL13-Rα2 antibody production. Methods for epitope mapping are known in the art and are described in, for example, U.S. Patent 5,747,240. Methods for isolating full-length IL13-Rα2 polypeptides are described in, for example, U.S. Patent 5,919,456.

[0020] In another embodiment, the invention provides a method for detecting an IL13-Rα2 polypeptide *in vitro* comprising (a) contacting a sample or cell suspected of containing IL13-Rα2 with an isolated antibody or fragment thereof that binds IL13-Rα2, and (b) detecting binding of the IL13-Rα2 antibody to IL13-Rα2. Any antibody (or fragment thereof) that binds IL13-Rα2, examples of which are set forth herein, is suitable for use in the inventive composition. The inventive method desirably employs an isolated antibody, or antigen-binding fragment thereof, that is directed against the full-length IL13-Rα2 or a fragment thereof. Isolated antibodies (or antibody fragments) that bind IL13-Rα2 have been developed and are available from a variety of sources, such as Cell Sciences, Inc. (www.cellsciences.com), and are described in, for example, Published U.S. Patent Application No. 20020197266/A1 and David et al., *Oncogene*, *20*, 6660-6668 (2001). Most preferably, the inventive method employs the IL13-Rα2 antibody (or antibody fragment) described herein, i.e., an antibody (or antibody fragment) which binds an epitope of an IL13-Rα2 comprising an amino acid sequence of SEQ ID NO:1, or consisting essentially of this sequence.

[0021] In one aspect of the inventive method a sample or cell suspected of containing IL13-Rα2 is contacted with an isolated antibody or fragment thereof that binds IL13-Rα2, and binding of the IL13-Rα2 antibody to IL13-Rα2 is detected. The sample or cell suspected of containing IL13-Rα2 can be isolated or derived from any tissue, organ, fluid (e.g., blood, lymph, or serum), or the like, from any suitable animal. A sample or cell is "derived" from a source when it is isolated from a source but modified in any suitable manner (e.g., by introduction of exogenous nucleic acid sequences, or modification of endogenous genomic DNA) so as not to disrupt the normal function of the source sample or cell. Thus, one of ordinary skill in the art will appreciate that the inventive method can be used to determine expression of IL13-Rα2 in a sample or cell at the cellular or subcellular

level, as well as the presence of soluble forms of IL13-Rα2 in a liquid sample (e.g., bodily fluid). The sample or cell preferably is isolated or derived from a mammal, most preferably a human. The sample or cell preferably is either a tissue sample isolated or derived from a mammal or is a cell grown in cell culture. In a particularly preferred embodiment, the sample or cell is isolated or derived from an organ, tissue, fluid, or the like, that is suspected to be affected by any disease caused by or associated with expression of IL13-Rα2. In this regard, the sample or cell preferably is isolated or derived from a solid tumor, such as, for example, an organ or tissue affected by malignant glioblastoma multiforme (GBM), anaplastic astrocytoma (AA), Kaposi sarcoma (KS), and renal cell carcinoma (RCC). The inventive method, however, is not limited to detecting IL13-Rα2 expression in these exemplary tumor types. The inventive method can be practiced using any sample or cell suspected of containing (i.e., expressing) an IL13-Rα2.

[0022] The sample or cell is contacted with an antibody (or antibody fragment) that binds IL13-Rα2 using any suitable method known in the art. Such methods can be *in vitro* or *in vivo*. Suitable *in vitro* methods for contacting the sample or cell include, include, for example, providing the antibody (or antibody fragment) to the culture medium in which the sample or cell is maintained or propagated. Alternatively, the antibody (or antibody fragment) can be provided by transfecting a culture of cells suspected of containing IL13-Rα2 with an expression vector comprising a polynucleotide sequence encoding the antibody (or antibody fragment), such that the polynucleotide is expressed and the antibody (or antibody fragment) is produced in the cell. In yet another alternative, lysates of cells suspected of containing IL13-Rα2 can be prepared using routine cell culture techniques and incubated with an antibody (or antibody fragment) that binds IL13-Rα2. With respect to *in vivo* contacting methods, one of ordinary skill in the art will appreciate that an antibody (or antibody fragment) can be formulated into a composition comprising a physiologically acceptable carrier and administered directly to an animal (e.g., a human) via numerous routes. Exemplary formulations, carriers, and administration routes for *in vivo* administration of an IL13-Rα2 antibody (or fragment thereof) are known in the art and described elsewhere herein. The present invention, however, is not limited to these exemplary *in vitro* and *in vivo* contacting methods. Any suitable method for contacting a sample or cell with an IL13-Rα2 antibody (or fragment thereof) is within the scope of the present invention.

[0023] Detecting binding of an IL13-Rα2 antibody to IL13-Rα2 can be performed using any suitable method to detect protein-protein, ligand-receptor, and/or antibody-antigen interactions. Such methods are well known to those skilled in the art, and include, for example, flow cytometry, ELISA, affinity chromatography, competitive inhibition assay, radioimmunoassay, immunofluorescence microscopy, immunoelectron microscopy,

immunocytochemistry (also referred to in the art as immunohistochemistry), and immunoprecipitation. Such methods are described in, for example, Janeway et al., *supra*, David et al., *supra*, Salvatore et al., *Biochem. Biophys. Res. Comm.*, *294*, 813-817 (2002), Berger et al., *Eur J Cell Biol.*, *67*, 106-11 (1995), Cechetto et al., *Exp Cell Res.*, *260*, 30-39 (2000), Gruber et al., *BMC Musculoskeletal Disorders*, *3*, 1-5 (2002), Harlow and Lane, *supra*, and U.S. Patents 4,661,913, 5,366,859, 5,491,096, and 5,958,715. The inventive method, however, should not be construed as being limited to these exemplary detection methods. Indeed, any method that can detect binding of an IL13-Rα2 antibody to IL13-Rα2 is within the scope of the present invention. One of ordinary skill in the art will appreciate that these exemplary methods also can enable the quantification of the amount of IL13-Rα2 expressed in a particular sample or cell, as well as the actual number of IL13-Rα2 receptors present in a particular sample or cell. Thus, the aforementioned methods for detecting IL13-Rα2 expression in a sample or cell also can be employed to quantify the number of IL13-Rα2 polypeptides that are present in the sample or cell. Most preferably, the number of IL13-Rα2 polypeptides in a sample or cell is quantified using flow cytometry-based applications.

[0024] The present invention also provides a method for localizing IL13-Rα2 in a sample or cell comprising (a) contacting the sample or cell with an isolated antibody that binds the IL13-Rα2, (b) detecting binding of the IL13-Rα2 antibody to the IL13-Rα2, and (c) determining the location of the IL13-Rα2 in the sample or cell. Descriptions of the antibody (or antibody fragment), the sample or cell, the detection methods, and components thereof set forth above in connection with other embodiments of the invention also are applicable to those same aspects of the aforesaid inventive method.

[0025] Methods for determining the location of (i.e., localizing) a polypeptide (e.g., a receptor) within a cell are known in the art, some of which are identical to those described herein for detecting ligand-receptor or antigen-antibody binding. Thus, in some embodiments of the invention, methods that detect binding of an IL13-Rα2 antibody (or antibody fragment) to IL13-Rα2 also will reveal the location of the IL13-Rα2 within the cell. Such methods preferably include, for example, immunofluorescence microscopy, immunoelectron microscopy, and immunocytochemistry. While electron microscopy provides higher resolution, light microscopy can provide sufficient spatial resolution in less time, and also can be used in connection with the inventive method. In other embodiments, detection of the IL13-Rα2 antibody (or antibody fragment) binding to IL13-Rα2 will not provide any information with respect to the location of the IL13-Rα2 in a sample or cell. In such cases, therefore, localization of the IL13-Rα2 must be determined separately from, and following, the detection of an IL13-Rα2 antibody (or antibody fragment) binding to IL13-Rα2.

[0026] As mentioned herein, expression (or overexpression) of IL13-Rα2 is associated with a number of pathological conditions, including certain types of cancer. Thus, the present invention further provides a method for diagnosing a disease characterized by expression of IL13-Rα2 comprising contacting a cell with an isolated antibody that binds IL13-Rα2, wherein the detectable binding of the IL13-Rα2 antibody indicates expression of the IL13-Rα2, and the disease is diagnosed. In this respect, methods described herein for detecting IL13-Rα2 expression in a sample or cell also can be used in connection with the aforementioned method for diagnosing a disease. Moreover, descriptions of the antibody (or antibody fragment), the sample or cell, and components thereof set forth above in connection with other embodiments of the invention also are applicable to those same aspects of the aforesaid inventive method. The inventive method can be used to diagnose any disease associated with or caused by IL13-Rα2 expression. In a particularly preferred embodiment, the inventive method is used to diagnose cancer in a patient. In this regard, the sample or cell preferably is a tumor cell. Most preferably, the sample or cell is derived from a malignant glioma, such as, for example, glioblastoma or anaplastic astrocytoma. Alternatively, the inventive method can be used to diagnose other cancers associated with or caused by IL13-Rα2 expression, such as, for example, Kaposi sarcoma (KS) or renal cell carcinoma (RCC).

[0027] The invention provides a method for killing a cell that expresses IL13-Rα2 comprising contacting the cell with an isolated antibody that binds to IL13-Rα2 and is conjugated to a cytotoxic agent, such that the IL13-Rα2 antibody binds IL13-Rα2 and the cytotoxic agent contacts the cell, whereby the cell is killed. Descriptions of the antibody (or antibody fragment), and components thereof, set forth above in connection with other embodiments of the invention also are applicable to those same aspects of the aforesaid inventive method. In this embodiment, the IL13-Rα2 antibody (or antibody fragment) can be used as a therapeutic agent to target and kill cells that express or overexpress an IL13-Rα2. Suitable target cells have been described herein, and include tumor cells such as malignant glioma cells (e.g., glioblastoma and anaplastic astrocytoma), Kaposi's sarcoma cells, and renal cell carcinoma cells. The inventive method, however, is not limited to these exemplary target cells. Indeed, cells derived from tissue affected by any disease associated with or caused by IL13-Rα2 expression can be targeted and killed in accordance with the inventive method, thereby preferably resulting in treatment of the disease. In an alternative embodiment, the IL13-Rα2 antibody can be used to treat conditions associated with IL13-induced inflammation, such as, for example, certain allergic conditions including asthma. In this regard, the inventive IL13-Rα2 antibody can be used as therapeutic agent to bind IL13-Rα2, thereby preventing IL13 from binding to the receptor. In this manner, signaling through the IL13-Rα2 is blocked, and IL13-mediated inflammation is inhibited.

[0028] The isolated antibody (or antibody fragment) preferably is conjugated to a cytotoxic agent. Any suitable cytotoxic agent that can be joined to the IL13-Rα2 can be used in practicing the present invention, so long as sufficient cytotoxicity is preserved in the ultimate conjugate molecule. The IL13-Rα2 antibody (or antibody fragment) and cytotoxic agent can be joined by any suitable means that provides for retention of the targeting and cytotoxicity characteristics of the IL13-Rα2 antibody (or antibody fragment) and cytotoxic agent, respectively. For example, the IL13-Rα2 antibody (or antibody fragment) and cytotoxic agent can be joined chemically such as through cysteine disulfide or other chemical conjugation methods. Desirably, the IL13-Rα2 antibody (or antibody fragment) and cytotoxic agent are joined at the genetic level in a recombinant fusion protein, such as is described in U.S. Patents 5,614,191 and 5,919,456.

[0029] Many cytotoxic molecules are known and are suitable for use as the cytotoxic agent. Suitable toxins include *Pseudomonas* exotoxin, ricin, *Diphtheria* toxin, abrin, a radionuclide (i.e., a radioisotope), and the like. Suitable cytotoxic agents maintain their cytotoxicity when joined with the IL13-Rα2 antibody. Derivatives of the cytotoxic agent, including genetic and chemical derivatives, are also suitable for use so long as sufficient cytotoxicity is preserved in the ultimate antibody-cytotoxic agent conjugate.

[0030] In a preferred embodiment of the invention, the IL13-Rα2 antibody (or antibody fragment) is introduced to human cells *in vivo*. In such applications, the method can be used alone or adjunctively as part of a treatment for any of a number of malignancies, such as those set forth above. For use *in vivo*, the antibody (or antibody fragment) desirably is formulated into a composition comprising a physiologically acceptable carrier. Any suitable physiologically acceptable carrier can be used within the context of the invention, and such carriers are well known in the art.

[0031] The carrier typically will be liquid, but also can be solid, or a combination of liquid and solid components. The carrier desirably is physiologically acceptable (e.g., a pharmaceutically or pharmacologically acceptable) carrier (e.g., excipient or diluent). Physiologically acceptable carriers are well known and are readily available. The choice of carrier will be determined, at least in part, by the location of the target tissue and/or cells, and the particular method used to administer the composition. In terms of using polypeptide therapeutics as active ingredients, the technology of U.S. Patents 4,608,251, 4,601,903, 4,559,231, 4,559,230, and 4,596,792, each incorporated herein by reference, can be used.

[0032] Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for using to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and the preparations can also be emulsified. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous

propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxycellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0033] The antibody for use in the present invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such as organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0034] The composition can further comprise any other suitable components, especially for enhancing the stability of the composition and/or its end-use. Accordingly, there is a wide variety of suitable formulations of the composition of the invention. The following formulations and methods are merely exemplary and are in no way limiting.

[0035] Formulations suitable for administration via inhalation include aerosol formulations. The aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also can be formulated as non-pressurized preparations, for delivery from a nebulizer or an atomizer.

[0036] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of a sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. In a preferred embodiment of the invention, the IL13-Rα2 antibody (or antibody fragment) is formulated for injection or

parenteral administration. In this regard, the formulation desirably is suitable for intratumoral administration, but also can be formulated for intravenous injection, intraperitoneal injection, subcutaneous injection, and the like.

[0037] Formulations suitable for anal administration can be prepared as suppositories by mixing the active ingredient with a variety of bases such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0038] In addition, the composition can comprise additional therapeutic or biologically-active agents. For example, therapeutic factors useful in the treatment of a particular indication can be present. Factors that control inflammation, such as ibuprofen or steroids, can be part of the composition to reduce swelling and inflammation associated with *in vivo* administration of the IL13-Rα2 antibody (or antibody fragment) and physiological distress. Immune system suppressors can be administered with the composition method to reduce any immune response to the antibody itself or associated with a disorder. Alternatively, immune enhancers can be included in the composition to upregulate the body's natural defenses against disease. Moreover, cytokines can be administered with the composition to attract immune effector cells to a disease (e.g., tumor) site.

[0039] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0040] This example demonstrates the generation of an isolated antibody directed against IL13-Rα2 that binds an epitope comprising an amino acid sequence of SEQ ID NO:1.

[0041] Immunogenic epitopes of the IL13-Rα2 receptor were identified using DNA sequence analysis and epitope mapping techniques known in the art and described herein. A nucleic acid sequence of SEQ ID NO:2 was identified as encoding an IL13-Rα2 epitope comprising an amino acid sequence of SEQ ID NO:1. An expression vector comprising SEQ ID NO:2 operatively linked to a CMV promoter was generated as described in WO 00/29444. Chickens of strain Hy-line SC (Hyline, Inc., Dallas Center, Iowa) were vaccinated by administration of the expression vector to chicken back skin using gene gun technology known in the art (see, e.g., WO 00/29444 and WO 01/88162).

[0042] Twenty days post immunization, 10 eggs from each immunized chicken were collected for antibody isolation. In this regard, IgY antibodies specific for the IL13-Rα2 epitope comprising SEQ ID NO:1 were isolated from egg yolks and purified as described in

Polson et al., *Immunol. Commun.*, *9*, 475-493 (1980) and in WO 01/88162 and WO 00/29444.

EXAMPLE 2

[0043] This example demonstrates the detection and localization IL13-Rα2 in a sample using the antibody of Example 1.

[0044] U251 human glioblastoma cells and normal control brain cells are cultured under standard conditions and metabolically labeled with [^{35}S] methionine as described in Harlow and Lane, *supra*. Cell lysates are prepared in and incubated with the antibody of Example 1. Beads coated with protein A purified from *S. aureus*, which binds to the Fc portion of an antibody, are added, and the beads are collected via centrifugation. In this manner, collection of the protein A beads results in purification of any antigen-antibody complexes ("immunoprecipitates") that have formed. The immunoprecipitates are washed and separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE) using methods known in the art. The gel is dried and visualized via autoradiography. Immunoprecipitation methods are described in detail in Harlow and Lane, *supra*.

[0045] Localization of IL13-Rα2 is performed using immunocytochemistry methods known to those skilled in the art. Briefly, U251 cells and control cells are fixed with formalin, and tissue sections are prepared. Tissue sections are incubated with the antibody of Example 1. The cells are washed with PBS and incubated with an anti-chicken secondary antibody conjugated to biotin. To detect biotinylated antibodies, the sections are incubated with streptavidin that is either fluorescently labeled or conjugated to a colorimetric enzyme, such as horseradish peroxidase. Antibody binding is visualized via fluorescence microscopy or light microscopy, depending on the secondary antibody used. Standard immunocytochemistry techniques are described in detail in, for example, Janeway et al., *supra*, and Gruber et al., *supra*.

EXAMPLE 3

[0046] This example demonstrates a method of killing a cell that expresses IL13-Rα2 comprising contacting the cell with an IL13-Rα2 antibody that is conjugated to a cytotoxic agent.

[0047] A fusion protein comprising the IL13-Rα2 antibody of Example 1 and a mutated and truncated form of *Pseudomonas* exotoxin is generated as described herein using standard molecular biology techniques (see, e.g., Sambrook et al., *supra*). Intratumoral injections of the antibody-exotoxin conjugate in concentrations of 50 and 100 μg/kg/day are administered for five consecutive days into nude mice having subcutaneous U251 glioblastoma tumors, resulting in a complete response (eradication of the tumor). Three

alternate day intratumoral injections of the antibody-exotoxin conjugate at a dose of 250 μg/kg/day into subcutaneous U87 glioblastoma tumors also produce a complete response in all mice.

[0048] A 25 or 50 μg/kg/dose of the antibody-exotoxin conjugate is administered to nude mice having U251 xenografts via intraperitoneal injection for five days, twice daily, resulting in tumor regression or complete response. A 50 μg/kg intraperitoneal injection into nude mice having U87 xenografts causes a reduction in the tumor burden to one-half. Likewise, daily intravenous injections of the antibody-exotoxin conjugate at doses of 25 and 50 μg/kg for five days suppresses the growth of subcutaneous U251 tumors or results in a complete response in the animals of each treatment group. The antibody-exotoxin treatment manifests no toxicity in any of the treated mice.

[0049] The IL13-Rα2 antibody-exotoxin conjugate is directly injected into glioblastoma multiforme tumors xenografted into the right caudate nucleus of nude rat brain. A single injection of 33.3 μg/kg of antibody-exotoxin conjugate into intracranial tumors increases the median survival by >20% compared to control rats.

EXAMPLE 4

[0050] This example demonstrates the sensitivity of the IL13-Rα2 antibody described in Example 1.

[0051] Evaluation of sensitivity of the test was performed on a combination of 29 human astrocytoma and renal cell carcinoma specimens. Formalin-fixed, paraffin-embedded tissues were used in this indirect IHC test using the biotinylated rabbit anti-IgY secondary antibody and the streptavidin-HRP tertiary reagent. Positive membranous and cytoplasmic staining is expected in tumor cells of human astrocytoma and renal cell carcinoma specimens, based on literature reports (see the list of citations at the conclusion of this Specification).

[0052] The immunohistochemistry was performed by first de-parrafinizing and re-hydrating the sections in graded alcohols. Heat-induced epitope retrieval then was performed using BORG buffer, 3 min., at 120 °C, 20 psi, followed by Trypsin (0.025% Trypsin in PBS), 1 minute. Following this treatment, the sections were blocked with perodxidase in 3% H_2O_2 in PBS for 15 minutes at room temperature. The sections then were washed 3 times for 3 minutes in TBST. Then, the sections were exposed to the anti IL1-3Rα2 IgY at 0.5 mg/ml (diluted in DAKO diluent), for 30 minutes at room temperature. Following exposure to the antibody, the sections were again washed 3 times for 3 minutes each in TBST. Next, the sections were blocked with a 5% blocking solution (in PBS) for 10 minutes at room temperature, which was tapped off prior to exposure to secondary antibody. The sections then were exposed to a biotinylated rabbit anti-IgY

secondary antibody (GenWay Biotech) at 10 mg/ml (diluted in DAKO diluent), for 15 minutes at room temperature. Following exposure to the secondary antibody, the sections were again washed 3 times for 3 minutes each in TBST. Next, the sections were exposed to streptavidin peroxidase (Pierce Chemical Co., Rockford, IL) at 0.5 U/ml (diluted in DAKO diluent), for 20 minutes at room temperature. Following this treatment, the sections were again washed 3 times for 3 minutes each in TBST. The sections then were treated with DAB (DakoCytomation, Carpinteria, CA) for 5 minutes at room temperature, following which, they were washed in deionized H_2O. Following the wash, the sections then were counterstained, dehydrated, and cover-slipped and observed via microscopy.

[0053] Adequate sensitivity was demonstrated by positive staining of the antibody in tumor cells of the astrocytoma and renal cell carcinoma specimens (see table 1). The acceptance criterion for the highest staining intensity is defined as greater than or equal to 10% of tumor cells staining positive at the specified intensity. Strong (3+) membranous and cytoplasmic staining was observed in 31% (9/29) of astrocytoma and renal cell carcinoma specimens, and moderate (2+) staining in 13.8% (4/29) of the specimens. Weak (1+) membranous and cytoplasmic staining was seen in 48.3% (14/29) of astrocytoma and renal cell carcinoma specimens. No staining was observed in 6.9% (2/29) of the specimens. The sensitivity of the test, shown as percentage of positive staining in astrocytoma and renal carcinoma specimens, is consistent with the expression of IL13-Rα2 in astrocytoma and renal cell carcinoma, based on literature reports (see the list of citations at the conclusion of this Specification).

Table 1

Antibody Validation

Sensitivity Worksheet

Antibody Name:	Affi-Anti-IL-13Rα2 IgY
Clone Name:	N/A

Target:	IL-13Rα2
Working Conc.:	10 ug/ml
Pretreatment:	HIER (BORG) 120°C, 3' + Trypsin 1'
Isotype:	Chicken IgY

General Specimen Information				Distinctive Tissue Element (Endothelium) % Cells Staining at Each Intensity							Long H-Score	Normal	Endo-thelium	Smooth Muscle	Fibro-blast	Stroma	Inflam. Cells	Nerve	Date Stained
	Specimen ID	Tissue Type	Background	3+	Sub	2+	Sub	1+	Sub	0									
Controls																			
C	ITKI02476B	Renal Cell CA	0	30	M,C	40	M,C	30	M,C	0	200	NS	±	NS	2+	±	0	NS	5/2/03
C	ITCC04367D	A172 Cell Line	0	90	C	10	C	0		0	290	NA	NA	NA	NA	NA	NA	NA	5/2/03
Samples																			
1	ITGL0107-185-00852-02	c/w Glioblastoma	0	0		0		70	C	30	70	NS	0	NS	NS	±	0	NS	5/2/03
2	ITGL0110-307-00779-02	c/w Astrocytoma[1]	0	0		0		100	C	0	100	NS	0	NS	NS	±	1+	NS	5/2/03
3	ITGL0110-307-02287-01	c/w Astrocytoma	0	0		0		100	C,M[2]	0	100	NS	±	NS	NS	±	NS	NS	5/2/03
4	ITGL0110-307-02291-02	c/w Astrocytoma	0	0		5	C	95	C	0	105	NS	±	NS	NS	±	NS	NS	5/2/03
5	ITGL0110-307-02297-01	c/w Astrocytoma	0	15	C,M	30	C,M	55	C	0	160	NS	NS	NS	NS	±	NS	NS	5/2/03
6	ITGL0110-307-02298-01	c/w Astrocytoma	0	0		0		100	C[3]	0	100	3+ C[4]	3+	NS	NS	±	NS	NS	5/2/03
7	ITGL0110-307-02300-02	c/w Astrocytoma	0	2	M,C	3	M,C	95	C	0	107	NS	0	NS	NS	0	NS	NS	5/2/03
8	ITGL0202-307-02141-01	c/w Astrocytoma	0	0		0		0		100	0	NS	1+	NS	NS	±	NS	NS	5/2/03
9	ITGL0202-307-02143-06	c/w Astrocytoma	0	5	M,C	5	M,C	90	C	0	115	0-1+ C	1+	NS	NS	±	NS	NS	5/2/03
10	ITGL0205-307-00709-02	c/w Astrocytoma	0	0		10	C,M	80	C	10	100	NS	0	1-2+	NS	1+	NS	NS	5/2/03
11	ITGL0205-292-00341-02	c/w Astrocytoma	0	0		10	C	60	C	30	80	NS	0	NS	NS	±	NS	NS	5/2/03
12	ITGL0110-307-00781-1	c/w Astrocytoma	0	15	M,C	50	C,M	35	C	0	180	NS	0-±	NS	NS	±	NS	NS	5/2/03
13	ITGL0204-307-00786-1	c/w Astrocytoma	0	0		0		100	C	0	100	NS	0-±	NS	NS	±-1+	0	NS	5/2/03
14	ITGL0205-292-00954-4	c/w Astrocytoma	0	20	C,M	30	C,M	50	C	0	170	0-1+ C	0-1+ C	NS	NS	1+	NS	NS	5/2/03
15	ITKI02472A	c/w Renal Cell CA	0	5	M,C	5	M,C	90	C	0	115	NS	1+	NS	2+	±	NS	NS	5/2/03
16	ITKI02474A	Renal Cell CA	0	10	M,C	20	M,C	70	C,M	0	140	NS	1+	NS	NS	±	0	NS	5/2/03

	General Specimen Information			Distinctive Tissue Element (Endothelium) % Cells Staining at Each Intensity							Long H-Score	Normal	Endo-thelium	Smooth Muscle	Fibro-blast	Stroma	Inflam. Cells	Nerve	Date Stained
	Specimen ID	Tissue Type	Background	3+	Sub	2+	Sub	1+	Sub	0									
17	ITKI02475A	Renal Cell CA	0	50	C	30	C	20	C	0	230	NS	0	NS	NS	±	0	NS	5/2/03
18	ITKI02477A	Renal Cell CA	0	30	C,M	20	C,M	50	C,M	0	180	NS	1+	NS	NS	±	NS	NS	5/2/03
19	ITKI02479A	Renal Cell CA	0	10	C	40	C	50	C	0	160	NS	1-2+	NS	NS	±	NS	NS	5/2/03
20	ITKI02480A	Renal Cell CA	0	0		0		100	C,M	0	100	NS	1-2+	NS	NS	0	NS	NS	5/2/03
21	ITKI02481A	Renal Cell CA	0	0		5	M,C	95	M,C	0	105	NS	1+	1-2+	NS	0	NS	NS	5/2/03
22	ITKI02482A	Renal Cell CA	0	20	C	50	C	30	C	0	190	NS	0-1+	1-2+	NS	0	NS	NS	5/2/03
23	ITKI02542A	Renal Cell CA	0	10	C	20	C	70	C	0	140	NS	0-1+	NS	NS	0	NS	NS	5/2/03
24	ITKI02544A	Renal Cell CA	0	0		5	M	95	M	0	105	NS	1-2+	1+	NS	0	NS	NS	5/2/03
25	ITKI02547A	Renal Cell CA	0	0		5	M,C	95	C	0	105	NS	1-2+	NS	2+	0	NS	NS	5/2/03
26	ITKI02548A	Renal Cell CA	0	0		2	C	98	M,C	0	102	NS	1+	NS	NS	±	NS	NS	5/2/03
27	ITKI02549A	Renal Cell CA	0	0		0		100	M	0	100	NS	2+	NS	NS	±	NS	NS	5/2/03
28	ITKI03601A	c/w Renal Cell CA	0	0		0		0		100	0	NS	0	NS	±	±	NS	NS	5/2/03
29	ITKI03621A	Renal Cell CA	0	0		5	M,C	85	M	10	95	NS	1-2+	2+C	NS	±	NS	NS	5/2/03
	Totals																		
Number of samples staining 3+ and > 9%				9															
Number of samples staining 0 at 100%				2															
Average H-Score of samples				115.7															

Comments:

[1] Tissue is mostly necrotic. Only small focus of viable tumor.

[2] Unusual cytoplasmic granular staining

[3] Very weak staining

[4] Choroid plexus

± = Equivocal Results	B = Basal Layer Staining	I = Inflammatory Cells	P = Perineural Staining
NA = Not Applicable	C = Cytoplasmic Staining	La = Luminal Accentuation	S = Stroma
NS = Not Seen	F = Focally Positive	M = Membrane Staining	Sc = Scattered
A = Apical Staining	H = Heterogeneous Staining	N = Nuclear Staining	Sub = Subcellular Localization

EXAMPLE 5

[0054] This example demonstrates the reactivity pattern of the IL13-Rα2 antibody described in Example 1.

[0055] Evaluation of the antibody reactivity pattern was performed in as selection of 30 normal human tissue types. The immunohistochemistry was performed as described in Example 4. For each tissue type, three specimens were tested. Positive membranous and cytoplasmic staining is expected in a variety of normal tissues, based on literature reports (see the list of citations at the conclusion of this Specification).

[0056] Differential staining of the antibody in a variety of normal human tissue specimens demonstrated adequate specificity (see Table 2). The acceptance criterion for the highest staining intensity is defined as greater than or equal to 10% of the distinctive tissue element staining positively at the specified intensity. Strong (3+) membranous and cytoplasmic staining was observed in tissue sections from: colon, kidney, and placenta. Moderate (2+) membranous and cytoplasmic staining was observed in tissue sections from bladder, bone marrow, breast, cervix, lung, lymph node, pancreas, parotid, pituitary, prostate, small intestine, stomach, testis, tonsil, ureter, and uterus. Weak (1+) cytoplasmic staining was observed in tissue sections from adrenal, esophagus, heart, liver, muscle, ovary, skin, spinal cord, and thyroid. Unusual coarse cytoplasmic granular straining was seen in adrenal, breast, cervix, colon, esophagus, kidney, pancreas, parotid, small intestine, stomach, thyroid, ureter, and uretus tissues. The significance of such staining is uncertain and the possibility of staining artifact cannot be excluded since coarse cytoplasmic staining is not characteristic for specific IHC staining of IL13Rα2. Furthermore, coarse cytoplasmic staining is not an uncommon staining artifact in IHC, generally. No staining was observed in brain and spleen. The staining pattern is consistent with expression of IL13Rα2 in normal tissues, based on literature reports (see the list of citations at the conclusion of this Specification).

Table 2

Antibody Validation

Specificity Worksheet

Antibody:	Affi-Anti-IL-13Rα2 IgY
Clone:	Polyclonal, Affinity Purified

Target:	IL-13Rα2
Working Conc.:	10 µg/ml
Pretreatment:	HIER (BORG) 120°C 3' followed by trypsin 1'
Isotype:	Chicken IgY

General Specimen Information				Staining of Distinctive Tissue Elements				Staining of Other Cell Types						
				% Cells Staining at Each Intensity										
Specimen ID#	Tissue Type	Histological Review	Isotype Control Background	3+	2+	1+	0	Endothelium	Smooth Muscle	Fibroblast	Stroma	Inflam. Cells	Nerve	Date Stained
ITKI02476B	Kidney CA	Kidney CA	0	20 M,C	50 M,C	30 C,M	0	2+ C	1+ C	NS	0-±	0-3+ M,C	NS	5/6/03
ITCC04367D	A172 Cell Line	Cell Line	0	50 C,M	50 C	0	0	NA	NA	NA	NA	NA	NA	5/6/03
ITKI02476B	Kidney CA	Kidney CA	0	20 C,M	40 C,M	40 C,M	0	0-±	±-1+	NS	±	0-2+	NS	5/15/03
ITCC04367D	A172 Cell Line	Cell Line	0	0	20 C	80 C	0	NA	NA	NA	NA	NA	NA	5/15/03
ITKI02476A	Kidney CA	Kidney CA	0	30 C,M	40 C,M	30 C,M	0	0-1+ C	NS	NS	±	0	NS	5/30/03
ITCC04367D	A172 Cell Line	Cell Line	0	0	60 C,M	40 C,M	0	NA	NA	NA	NA	NA	NA	5/30/03
ITKI02476B	Kidney CA	Renal Cell CA	0	50 C,M	20 M,C	30 C,M	0	0	NS	2+ C	±	0	NS	6/10/03
ITCC04367D	A172 Cell Line	Cell Line	0-1+ C	0	50 C	50 C	0	NA	NA	NA	NA	NA	NA	6/10/03
INAD02614A	Adrenal	c/w Adrenal	0	0	5 C^1	60 C^1	35	±	1-2+ C,F	NS	0-1+ F	0	0	5/15/03
INAD03644A	Adrenal	Adrenal	1+	0	0	50 C^1	50	±	2+ C	NS	±	0	2+	5/15/03

Specificity (continued)

General Specimen Information				Staining of Distinctive Tissue Elements				Staining of Other Cell Types						
Specimen ID#	Tissue Type	Histological Review	Isotype Control Back-ground	% Cells Staining at Each Intensity				Endo-thelium	Smooth Muscle	Fibro-blast	Stroma	Inflam. Cells	Nerve	Date Stained
				3+	2+	1+	0							
INAD03648A	Adrenal	Adrenal	1+	0	0	70 C[1]	30	±	2+ C	NS	±	NS	NS	5/15/03
INBL01846A	Bladder	Bladder	0	0	30 C	70 C	0	±	2+ C	NS	±	1-3+ M,C	NS	5/15/03
INBL02983A	Bladder	Bladder	0	0	0	100 M,C	0	0	2+ C	NS	0	NS	NS	5/15/03
INBL02997A	Bladder	Bladder	0	0	30 C,M	70 C,M	0	0-±	2+ C	NS	0	NS	NS	5/15/03
INBO0111-307-00273-5	Bone Marrow	Bone Marrow	0	5 M,C	5 M,C	5 M,C	85	NS	NS	NS	0	NS	NS	5/30/03
INBO0103-212-01323-1	Bone Marrow	Bone Marrow	0	0	0	0	100	NS	NS	NS	0	NS	NS	5/30/03
INBO0107-305-02724-1	Bone Marrow	Bone Marrow	0	5 C	2 C	2 C	91	NS	NS	NS	±	NS	NS	5/30/03
INBA02029B	Brain	Brain (Cortex)	0	0	0	5 C	95	0	NS	NS	1+[2]	NS	NS	5/15/03
INCL02032A	Cerebellum	Brain (Cerebellum)	0	0	0	0	100[3]	±	NS	NS	1+[2]	NS	NS	5/15/03
INCL02035A	Cerebellum	Brain (Cerebellum)	0	0	0	0	100[3]	±	NS	NS	1+[2]	NS	NS	5/15/03
INBR01201A	Breast	Breast	0	0	0	100 C	0	NS	NS	NS	0	0-2+	NS	5/15/03
INBR01204A	Breast	Breast	0	0	80 C[1]	20 C[1]	0	±	NS	NS	0	0-2+	NS	5/15/03
INBR03066A	Breast	Breast	1+ C[1]	0	100 C[1]	0	0	1+ C	NS	NS	±	0-1+	NS	6/10/03
INCE00324C	Cervix	Cervix	0	0	0	100 M,C	0	0	1+	NS	0	NS	NS	5/15/03
INCE02778A	Cervix	Cervix	0	0	50 C	50 C	0	±	NS	NS	0-±	1-3+ M,C	NS	5/15/03
INCE0103-212-01579-1A	Cervix	Cervix	0-1+ C,F	0	20 C[1]	80 C,M	0	±	1-2+ C	NS	0	NS	NS	5/15/03
INCO03165A	Colon	Colon	0	0	30 C[1]	70 C[1]	0	0-±	2+ C	NS	0	0-3+ M,C	NS	5/15/03

General Specimen Information				Staining of Distinctive Tissue Elements				Staining of Other Cell Types						
Specimen ID#	Tissue Type	Histological Review	Isotype Control Back-ground	% Cells Staining at Each Intensity				Endo-thelium	Smooth Muscle	Fibro-blast	Stroma	Inflam. Cells	Nerve	Date Stained
				3+	2+	1+	0							
INCO03179A	Colon	Colon	0	100 C[1]	0	0	0	0-±	1-2+ C	NS	0	0-2+ M,C	NS	5/15/03
INCO03181D	Colon	Colon	0	0	80 C[1]	20 C[1]	0	0-±	1-2+ C	NS	0	0-1+ M,C	NS	5/15/03
INES01710A	Esophagus	Esophagus	0	0	0	100 C	0	0	2+ C	NS	0	0-2+ M,C	NS	5/15/03
INES01770A	Esophagus	Esophagus	0	0	0	100 C	0	±	3+ C[3]	NS	0	0	NS	5/15/03
INES01772A	Esophagus	Esophagus	0	0	0	100 C	0	0	1-2+ C	NS	0	NS	NS	5/15/03
INHE01517A	Heart	Heart	0	0	0	100 C	0	0	2+ C	NS	0	NS	NS	5/15/03
INHE01520A	Heart	Heart	0	0	0	0	100	0	2+ C	NS	0	NS	NS	5/15/03
INHE05535A	Heart	Heart	0	0	0	100 C	0	±-1+ C	2+ C	NS	0	NS	NS	5/30/03
INKI00956A	Kidney	Kidney	0	90 C[1]	0	0	10	0-1+	NS	NS	0	NS	NS	5/30/03
INKI00323J	Kidney	Kidney	0	50 C[1]	30 C[1]	10 C[1]	10	±	2+ C	NS	0	0	NS	5/30/03
INKI00968A	Kidney	Kidney	0	30 C[1]	10 C[1]	50 C[1]	10	±-1+	2+ C	NS	0	0	NS	5/30/03
INLI02224A	Liver	Liver	0	0	0	80 C[1]	20	1+ C	1+ C	NS	0	0-3+ M,C	NS	5/6/03
INLI03366A	Liver	Liver	0	0	0	100 C[1]	0	±	1+ C	NS	0	0-3+ M,C	NS	5/6/03
INLI03372A	Liver	Liver	0	0	0	60 C[1]	40	±	1+ C	NS	0	0-3+ M,C	NS	5/6/03
INLU02148A	Lung	Lung	0	0	0	100 C	0	1+ C	1+ C	NS	0-±	0-3+ M,C	NS	5/6/03
INLU01551A	Lung	Lung	0	0	0	80 C	20	1+ C	1+ C	NS	0-±	0-3+ C	NS	5/6/03

Specificity (continued)

General Specimen Information				Staining of Distinctive Tissue Elements				Staining of Other Cell Types						
Specimen ID#	Tissue Type	Histological Review	Isotype Control Background	% Cells Staining at Each Intensity				Endothelium	Smooth Muscle	Fibroblast	Stroma	Inflam. Cells	Nerve	Date Stained
				3+	2+	1+	0							
INLU01506A	Lung	Lung	0-±	0	10 C	90 C	0	1+ C	1+ C	NS	±	0-3+ M,C	NS	5/6/03
INLN02925A	Lymph Node	Lymph Node	0	5 M,C	5 M,C	20 C[5]	70	2+ M,C	1-2+ C	NS	±	NS	NS	5/6/03
INLN01624D	Lymph Node	Lymph Node	0	1 M,C	1 M,C	10 C[5]	88	±	1+ C	NS	0-±	NS	NS	5/6/03
INLN01620A	Lymph Node	Lymph Node	0	5 M,C	3 M,C	20 C[5]	72	3+ M	2+ C	NS	±-1+ F	NS	NS	5/6/03
INMU01512A	Muscle	Skeletal Muscle	0	0	0	50 C	50	1+ C	1+ C	NS	±	NS	NS	5/6/03
INMU01513A	Muscle	Skeletal Muscle	0	0	0	0	100	2-3+ M,C	1-2+ C,F	NS	NS	NS	NS	5/6/03
INMU0110-307-00822-8	Muscle	Skeletal Muscle	0	0	0	0	100	2+ M,F	1-2+ C,F	NS	0	NS	NS	5/6/03
INOV04286A	Ovary	Ovary	0	0	0	100 C	0	3+ M	1-2+ C,F	NS	±	NS	NS	5/6/03
INOV04320A	Ovary	Ovary	0	0	0	100 C	0	0-±	1+ C	NS	±	NS	NS	5/6/03
INOV04321A	Ovary	Ovary	0	0	0	100 C	0	2-3+ M	1-2+ C	NS	±	NS	NS	5/6/03
INPN01215A	Pancreas	Pancreas	0-3+ C,F[6]	0	50 C[1]	50 C[1]	0	1+ C	1-2+ C,F	NS	±	0-3+ C,M	0	5/6/03
INPN01503A	Pancreas	Pancreas	0-3+ C,F[6]	0	60 C[1]	40 C[1]	0	±	1+ C	NS	0-±	NS	NS	5/6/03
INPN0203-307-00245-2	Pancreas	Pancreas	0-3+ C,F	0	40 C[1]	60 C[1]	0	1+ C	1+ C	NS	0	NS	NS	5/6/03
INPO04089A	Parotid	Parotid	0-1+ C,F[7]	0	10 C[7,1]	50 C[1]	40	3+ M,C	1+ C	NS	0-±	NS	0-±	5/6/03
INPO0105-303-0818-6A	Parotid	Parotid	0-1+ C,F[7]	0	10 C[7,1]	0	90	3+ M,C	1+ C,F	NS	0-±	0-3+ M,C	NS	5/6/03
INPO0109-192-00553-1	Parotid	Parotid	0-1+ C,F[7]	0	20 C[1]	80 C[1]	0	0-±	1+ C	NS	0-±	0-3+ M,C	NS	5/6/03

Specificity (continued)

General Specimen Information				Staining of Distinctive Tissue Elements				Staining of Other Cell Types						
Specimen ID#	Tissue Type	Histological Review	Isotype Control Back-ground	% Cells Staining at Each Intensity				Endo-thelium	Smooth Muscle	Fibro-blast	Stroma	Inflam. Cells	Nerve	Date Stained
				3+	2+	1+	0							
INPI03653A	Pituitary	Pituitary	0-3+ C[8]	0	1 C	99 C	0	1-2+ C	1-2+ C	NS	±-1+ F	NS	NS	5/6/03
INPI03655A	Pituitary	Pituitary	0-2+ C[8]	0	0	100 C	0	0	1+ C	NS	±	NS	NS	5/6/03
INPI03656A	Pituitary	Pituitary	0-3+ C,F[8]	0	20 C	80 C	0	2+ C	NS	NS	±	NS	NS	5/6/03
INPL03550A	Placenta	Placenta	0	90 M,C	10 M,C	0	0	1-2+ C	0-1+ C	NS	0-±	NS	NS	5/6/03
INPL03554A	Placenta	Placenta	0	80 M,C	20 M,C	0	0	2+ M,C	1+ C	NS	±	NS	NS	5/6/03
INPL03555A	Placenta	Placenta	0	70 M,C	30 M,C	0	0	2-3M,C	1+ C	NS	0-±	NS	NS	5/6/03
IBPR00935A	Prostate	Prostate	0	0	30 C	70 C	0	1-2+	1+ C	NS	±	NS	NS	5/6/03
INPR02945A	Prostate	Prostate	0	0	50 C	50 C	0	3+ M,C	1+ C	NS	±-1+	3+ M,C	NS	5/6/03
INPR02960A	Prostate	Prostate	0	0	40 C	60 C	0	3+ M,C	1+ C	NS	0-1+ F	3+ M,C	NS	5/6/03
INSK01361L	Skin	Skin	0	0	0	100 C	0	1+ C	NS	NS	0	0	NS	5/30/03
INSK01342B	Skin	Skin	0	0	0	100 C	0	±	3+ C	NS	0	NS	NS	5/30/03
INSK01339T	Skin	Skin	0	0	0	100 C	0	NS	2+ C	NS	0	NS	NS	5/30/03
INSM03230A	Small Intestine	Small Intestine	0	0	0	100[9]	0	0	2+ C	NS	0	NS	NS	5/15/03
INDU00182B	Small Intestine	Small Intestine	0-±	0	15 C[1]	85 C[1]	0	0	1-2+ C	NS	0	0-1+ C	NS	5/15/03
INJE00181B	Small Intestine	Small Intestine	0	0	0	100 C	0	0	1-2+ C	NS	0	0-3+ C,M	NS	5/15/03
INSC03662A	Spinal Cord	Spinal Cord	0	0	0	100 C	0	±	2+ C	NS	0	0-1+ C	NS	5/15/03
INSC03664A	Spinal Cord	Spinal Cord	0	0	0	0	100	0	2+ C	NS	0	NS	0-±	5/15/03

Specificity (continued)

General Specimen Information				Staining of Distinctive Tissue Elements				Staining of Other Cell Types						
Specimen ID#	Tissue Type	Histological Review	Isotype Control Back-ground	% Cell Staining at Each Intensity				Endo-thelium	Smooth Muscle	Fibro-blast	Stroma	Inflam Cells	Nerve	Date Stained
				3+	2+	1+	0							
INSC03666A	Spinal Cord	Spinal Cord	0	0	0	0	100	0	2+ C	NS	±-1+[2]	NS	0	5/15/03
INSP02772B	Spleen	Spleen	0	0	0	0	100	0	NS	NS	0-1[2]	NS	NS	5/15/03
INSP03695A	Spleen	Spleen	0[10]	0	0	0	100	0	2+ C	NS	0-1+	NS	NS	5/15/03
INSP03699A	Spleen	Spleen	0	0	0	0	100	0	1+ C	NS	0-±	NS	NS	5/15/03
INST02911B	Stomach	Stomach	0	0	0	0	100	0	1-2+ C	NS	0	NS	NS	5/15/03
INST03697A	Stomach	Stomach	0	0	20 C[1]	80 C[1]	0	0	1-2+ C	NS	0	0-3+ C	NS	5/15/03
INST0103-212-01588-1	Stomach	Stomach	0	0	35 C[1]	65 C[1]	0	0	1-2+ C	NS	0	0-1+	NS	5/15/03
INTE04514A	Testis	Testis	0	0	80 C	20 C	0	0	1+ C	NS	0	NS	NS	5/15/03
INTE04516A	Testis	Testis	0	0	0	90 C	10	0	1+ C	NS	0	NS	NS	5/15/03
INTE04517A	Testis	Testis	0	0	80 C	20 C	0	NS	NS	NS	0	NS	NS	5/15/03
INTH03667A	Thyroid	c/w Thyroid	0-±	3 C[1]	2 C[1]	0	95	0	1+ C	NS	0	0-3+ M,C	NS	5/15/03
INTH03669A	Thyroid	Thyroid	0	0	0	0	100	0	2+ C	NS	0	NS	NS	5/15/03
INTH03671A	Thyroid	Thyroid	0	2 C[1]	0	98 C[1]	0	0	2+ C	NS	±	NS	NS	5/15/03
INTO03508A	Tonsil	Tonsil	0	0	0	0	100	1+ C	NS	NS	±	NS	NS	5/30/03
INTO03507A	Tonsil	Tonsil	0	0	0	0	100	1+	NS	NS	0	NS	NS	5/30/03
INTO03449A	Tonsil	Tonsil	0	5 M,C	5 M,C	10 C	80	0	2+ C	NS	0	NS	NS	5/30/03
INUR03677A	Ureter	Ureter	0	0	90 C[1]	10 C[1]	0	±	2+ C	NS	0-±	NS	NS	5/15/03
INUR03685B	Ureter	Ureter	0	0	0	100 C[1]	0	0	2+ C	NS	0	NS	NS	5/15/03
INUR05530A	Ureter	Ureter	0	0	0	100 C	0	0-±	2+ C	NS	0	NS	NS	5/30/03
INUT01842A	Uterus	Uterus	0	0	0	100 C[1]	0	0	1-2+ C	NS	0	NS	NS	5/15/03

General Specimen Information				Staining of Distinctive Tissue Elements				Staining of Other Cell Types						
Specimen ID#	Tissue Type	Histological Review	Isotype Control Background	% Cells Staining at Each Intensity				Endothelium	Smooth Muscle	Fibroblast	Stroma	Inflam. Cells	Nerve	Date Stained
				3+	2+	1+	0							
INUT0105-306-0054-11	Uterus	Uterus	0	0	0	100 C[1]	0	0	1-2+ C	NS	0	NS	NS	5/15/03
INUT0103-212-01579-2	Uterus	Uterus	0	0	25 C[1]	75 C[1]	0	0	1+ C	NS	0	NS	NS	5/15/03

Comments/Notes:

[1] Cytoplasmic granular staining of undetermined significance
[2] Neuropil
[3] Diffuse precipitate not cell-associated (between cells)
[4] Endocervical epithelium show membrane staining; squamous epithelium show cytoplasmic, granular staining.
[5] Sinus histiocytes are 1+ C.
[6] Rare strongly positive cells of undetermined significance
[7] Ductal epithelium is positive.
[8] Rare, (<1%) scattered positive cells
[9] Weak staining only
[10] Scattered hemosiderin pigment present in tissue sample

± = Equivocal Results	H = Heterogeneous Staining
NA = Not Applicable	I = Inflammatory Cells
NS = Not Seen	La = Luminal Accentuation
Ap = Apical Staining	M = Membrane Staining
B = Basal Layer Staining	N = Nuclear Staining
C = Cytoplasmic Staining	P = Perineural Staining
F = Focally Positive	S = Stroma
c/w = Consistent With	Sc = Scattered

WO 2004/087758 PCT/US2004/009354

EXAMPLE 6

[0057] This example demonstrates the precision of the IL13-Rα2 antibody described in Example 1.

[0058] Precision analysis was performed on 3 human astrocytoma specimens and 1 human renal cell carcinoma specimen on five different days under the same test conditions. The immunohistochemistry was performed as described in Example 4. Adequate precision was demonstrated by a generally consistent staining pattern and intensity of the tissues tested on five different days (See Table 3).

Table 3

Antibody Validation

Precision Worksheet

Antibody:	Affi-Anti-IL-13Rα2 IgY	Target:	IL-13Rα2
Clone:	Polyclonal, Affinity Purified	Working Conc.:	10 µg/ml
		Pretreatment:	HIER (BORG) 120°C 3' followed by trypsin 1'
		Isotype:	Chicken IgY

General Specimen Information				Staining of Distinctive Tissue Elements				Date Stained
Specimen ID#	Tissue Type	Histological Review	Isotype Control Background	% Cells Staining at Each Intensity				
				3+	2+	1+	0	
Day 1								
ITKI02476B	Kidney CA	Kidney CA	0	20 C,M	40 C,M	40 C,M	0	5/15/03
ITCC04367D	A172 Cell Line	Cell Line	0	0	20 C	80 C	0	5/15/03
ITGL0110-307-02312-1	Glioblastoma	c/w Glioblastoma	0-±	15 C,M	40 C	35 C	10	5/15/03
ITGL0110-307-02305-1	Glioblastoma	c/w Glioblastoma	0-±	0	0	90 C	10	5/15/03
ITGL0110-307-02285-1	Glioblastoma	c/w Glioblastoma	0	0	30 C	70 C	0	5/15/03
ITKI02473A	Renal Cell CA	c/w Renal Cell CA	±	30 M,C	30 M,C	30 C	10	5/15/03
Day 2								
ITKI02476B	Kidney CA	Kidney CA	0	20 C,M	50 C,M	30 C	0	5/20/03
ITCC04367D	A172 Cell Line	Cell Line	0	5 C	10 C	85 C	0	5/20/03
ITGL0110-307-02312-1	Glioblastoma	c/w Glioblastoma	0	5 C	30 C	65 C	0	5/20/03
ITGL0110-307-02305-1	Glioblastoma	c/w Glioblastoma	0	20 C,M	10 C	10 C	60	5/20/03
ITGL0110-307-02285-1	Glioblastoma	c/w Glioblastoma	0	2 C	70 C	28 C	0	5/20/03
ITKI02473A	Kidney CA	Kidney CA	0	10 C,M	10 C,M	80 M,C	0	5/20/03

General Specimen Information				Staining of Distinctive Tissue Elements				Date Stained
Specimen ID#	Tissue Type	Histological Review	Isotype Control Background	% Cells Staining at Each Intensity				
				3+	2+	1+	0	
Day 3								
ITKI02476A	Kidney CA	Kidney CA	0	30 C,M	40 C,M	30 C,M	0	5/30/03
ITCC04367D	A172 Cell Line	Cell Line	0	0	60 C,M	40 C,M	0	5/30/03
ITGL0110-307-02312-1	Glioblastoma	c/w Glioblastoma	0	10 C	40 C	50 C	0	5/30/03
ITGL0110-307-02305-1	Glioblastoma	c/w Glioblastoma	0	0	10 C	80 C	10	5/30/03
ITGL0110-307-02285-1	Glioblastoma	c/w Glioblastoma	0	0	20 C	80 C	0	5/30/03
ITKI02473A	Kidney CA	c/w Renal Cell CA	0	20 M,C	40 M,C	30 M,C	10	5/30/03
Day 4								
ITKI02476A	Kidney CA	Kidney CA	0	30 C,M	30 C,M	40 C,M	0	6/2/03
ITCC04367D	A172 Cell Line	Cell Line	0	0	10 C	90 C	0	6/2/03
ITGL0110-307-02312-1	Glioblastoma	c/w Glioblastoma	0	10 C	30 C	50 C	10	6/2/03
ITGL0110-307-02305-1	Glioblastoma	c/w Glioblastoma	0	0	20 C	80 C	0	6/2/03
ITGL0110-307-02285-1	Glioblastoma	c/w Glioblastoma	0	0	5 C	95 C	0	6/2/03
ITKI02473A	Kidney CA	c/w Kidney CA	0	20 C	40 C	30 C	10	6/2/03
Day 5								
ITKI02476B	Kidney CA	Renal Cell CA	0	50 C,M	20 M,C	30 C,M	0	6/10/03
ITCC04367D	A172 Cell Line	Cell Line	0-1+ C	0	50 C	50 C	0	6/10/03
ITGL0110-307-02312-1	Glioblastoma	c/w Glioblastoma	0	30 C	20 C	40 C	10	6/10/03
ITGL0110-307-02305-1	Glioblastoma	c/w Glioblastoma	0	40 C	20 C	40 C	0	6/10/03
ITGL0110-307-02285-1	Glioblastoma	c/w Glioblastoma	0	0	10 C	90 C	0	6/10/03
ITKI02473A	Kidney CA	c/w Kidney CA	0	30 C	40 C	30 C	0	6/10/03

Comments/Notes:

± = Equivocal Results	H = Heterogeneous Staining
NA = Not Applicable	I = Inflammatory Cells
NS = Not Seen	La = Luminal Accentuation
Ap = Apical Staining	M = Membrane Staining
B = Basal Layer Staining	N = Nuclear Staining
C = Cytoplasmic Staining	P = Perineural Staining
F = Focally Positive	S = Stroma
c/w = Consistent With	Sc = Scattered

WO 2004/087758 PCT/US2004/009354

EXAMPLE 7

[0059] This example compares RT-PCR to IHC using the IL13-Rα2 antibody described in Example 1.

[0060] A method comparison study, comparing RT-PCR to immunohistochemistry, was performed on 5 formalin-fixed, paraffin-embedded human glioblastoma specimens with adjacent tumor preserved for RNA extraction. The immunohistochemistry was performed as described in Example 4. Following RNA extraction, quantitative RT-PCR was performed on each specimen to determine the expression of the IL13-Rα2 gene. It is expected that IL13-Rα2 message will be presented in specimens that exhibit positive IHC staining.

[0061] Adequate method comparison was demonstrated by a qualitative agreement between the IHC staining pattern and intensity and the RT-PCR gene expression of the specimens. (See Table 4). Although there appears to be a general concordance between the two methods, a quantitative comparison between the results of the two methods does not show a high level of correlation. Factors to be considered in such a comparison include 1) the level of IL13-Rα2 mRNA may not directly correlate with protein expression, 2) the adjacent tumor specimens may have a different density of tumor cells, and 3) while RT-PCR is a true scalar quantity, immunohistochemistry is, at best, semi-quantitative. Considering all the factors, the method comparison of IL13-Rα2 expression between RT-PCR and IHC appears adequate.

Table 4

Antibody Validation

Method Comparison Study

General Specimen Information				IHC Staining of Distinctive Tissue Elements[1]				RT-PCR Results[2]	
				% Cells Staining at Each Intensity					
Specimen ID#	Tissue Type	Histological Review	Isotype Control, Background	3+	2+	1+	0	Mean Quantity IL-13Rα2 (copy #) / 50 ng RNA	Mean Quantity Actin (copy #) / 50 ng RNA
ITKI02476B	Kidney CA	Kidney CA	0	20 C,M	50 C,M	30 C	0	NA	NA
ITCC04367D	A172 Cell Line	Cell Line	0	5 C	10 C	85 C	0	NA	NA
02-703306	Brain Tumor	c/w Glioblastoma	2+ C	90 C	10 C	0	0	8103.30	1822.35
03-701458	Brain Tumor	c/w Glioblastoma	1+ C	40 C	20 C	25 C	15	6056.49	933.15
03-701460	Brain Tumor	c/w Glioma	0-1+ F	20 M,C	20 M,C	30 C,M	30	1877.30	476.85
03-701462	Brain Tumor	c/w Mixed Glioma	0-2+ C	30 M,C	20 M,C	10 C	40	1037.51	5140.10
03-701411	Brain Tumor	c/w Glioblastoma	0-2+ C	60 C,M	20 C,M	20 C,M	0	2303.73	7364.55

± = Equivocal Results	H = Heterogeneous Staining
NA = Not Applicable	I = Inflammatory Cells
NS = Not Seen	La = Luminal Accentuation
Ap = Apical Staining	M = Membrane Staining
B = Basal Layer Staining	N = Nuclear Staining
C = Cytoplasmic Staining	P = Perineural Staining
F = Focally Positive	S = Stroma
c/w = Consistent With	Sc = Scattered

[0062] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0063] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0064] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein, including the following:

Bernard et al., *Lab. Invest., 81*(9), 1223 (2001)

Daines et al., *J. Biol. Chem., 277*(12), 10387-93 (2002)

Daput et al., *J. Biol. Chem., 271*(28), 16921-26 (1996)

David et al., *Oncogene, 20(*46), 6660-68 (2001)

Debinski et al., *Clin. Canc. Res., 5*, 985-90 (1999)

Donaldson et al., *J. Immunol., 161*, 2317-24 (1998)

Feng et al., *Lab. Invest., 78*(5), 591-602 (1998)

Joshi et al., *Canc. Res., 60*, 1168-72 (2000)

Joshi et al., *Clin. Canc. Rres., 8(*6), 1948-56 (2002)
Kawakami et al., *Blood, 97*(9), 2673-79 (2001)
Kawakami et al., *Int. J. Cancer, 103*(1), 45-52 (2003)
Kawakami et al., *J. Immunol., 169*(12), 7119-26 (2002)
Liu et al., *Cancer Immunol. Immunother., 49*(6), 319-424 (2000)
Maini et al., *J. Urol., 158* (3Pt1), 948-53 (1997)
Rahaman et al., *Canc. Res., 62*, 11-3-09 (2002)
Tang et al., *Vet. Immunol. Immunopathol., 79*(3-4), 181-95 (2001)
Vita et al., *J. Biol. Chem., 270*(8), 3512-17 (1995)
Wu et al., *J. Neurooncol., 59*(2), 99-105 (2002)

WHAT IS CLAIMED IS:

1. An isolated antibody or antigen-binding fragment thereof directed against an interleukin 13 receptor alpha 2 (IL13-Rα2) that binds an epitope comprising an amino acid sequence of SEQ ID NO:1.

2. An isolated antibody or antigen-binding fragment thereof directed against an IL13-Rα2 that binds an epitope consisting essentially of an amino acid sequence of SEQ ID NO:1.

3. The antibody of claim 1 or 2, wherein the epitope is encoded by a nucleic acid sequence comprising SEQ ID NO:2.

4. The antibody of any of claims 1-3, wherein the antibody is a monoclonal antibody.

5. The antibody of any of claims 1-4, wherein the antibody is selected from the group consisting of a chicken antibody, a mouse antibody, a human antibody, and a humanized antibody.

6. The antibody of any of claims 1-5, wherein the antibody is a chicken IgY antibody.

7. The antibody of any of claims 1-6, wherein the antibody is an Fab fragment, an $(Fab')_2$ fragment, or a single chain Fv fragment.

8. The antibody of any of claims 1-7, wherein the antibody is conjugated to a cytotoxic agent.

9. The antibody of claim 8, wherein the cytotoxic agent is selected from the group consisting of a *Pseudomonas* exotoxin, a *Diphtheria* toxin, ricin, abrin, and a radionuclide.

10. A composition comprising the antibody of any of claims 1-9 and a physiologically acceptable carrier.

11. A method for detecting an IL13-Rα2 polypeptide *in vitro* comprising (a) contacting a sample or cell suspected of containing the IL13-Rα2 with an isolated antibody or fragment thereof that binds the IL13-Rα2, and (b) detecting binding of the IL13-Rα2 antibody to the IL13-Rα2.

12. The method of claim 11, wherein the antibody binds an epitope comprising an amino acid sequence of SEQ ID NO:1.

13. The method of claim 11, wherein the antibody binds an epitope consisting essentially of an amino acid sequence of SEQ ID NO:1.

14. The method of any of claims 11-13, wherein the antibody is a monoclonal antibody.

15. The method of any of claims 11-14, wherein the antibody is selected from the group consisting of a chicken antibody, a mouse antibody, a human antibody, and a humanized antibody.

16. The method of any of claims 11-15, wherein the antibody is a chicken IgY antibody.

17. The method of any of claims 11-16, wherein the detecting is by flow cytometry, enzyme linked immunosorbent assay (ELISA), affinity chromatography, competitive inhibition assay, radioimmunoassay, immunofluorescence microscopy, immunoelectron microscopy, immunocytochemistry, or immunoprecipitation.

18. A method for localizing an IL13-Rα2 in a sample or cell comprising (a) contacting the sample or cell with an isolated antibody or fragment thereof that binds the IL13-Rα2, (b) detecting binding of the antibody to the IL13-Rα2, and (c) determining the location of the IL13-Rα2 in the sample or cell.

19. The method of claim 18, wherein the antibody binds an epitope comprising an amino acid sequence of SEQ ID NO:1.

20. The method of claim 18, wherein the antibody binds an epitope consisting essentially of an amino acid sequence of SEQ ID NO:1.

21. The method of any of claims 18-20, wherein the antibody is a monoclonal antibody.

22. The method of any of claims 18-21, wherein the antibody is selected from the group consisting of a chicken antibody, a mouse antibody, a human antibody, and a humanized antibody

23. The method of any of claims 18-22, wherein the antibody is a chicken IgY antibody.

24. The method of any of claims 18-23, wherein the detecting and determining are by immunofluorescence microscopy, immunoelectron microscopy, or immunocytochemistry.

25. A method for diagnosing a disease characterized by expression of an IL13-Rα2 comprising contacting a sample or cell with an isolated antibody or fragment thereof that binds the IL13-Rα2, wherein the detectable binding of the IL13-Rα2 antibody indicates expression of the IL13-Rα2, and the disease is diagnosed.

26. The method of claim 25, wherein the antibody binds an epitope comprising an amino acid sequence of SEQ ID NO:1.

27. The method of claim 25, wherein the antibody binds an epitope consisting essentially of an amino acid sequence of SEQ ID NO:1.

28. The method of any of claims 25-27, wherein the antibody is a monoclonal antibody.

29. The method of any of claims 25-28, wherein the antibody is selected from the group consisting of a chicken antibody, a mouse antibody, a human antibody, and a humanized antibody.

30. The method of any of claims 25-29, wherein the antibody is a chicken IgY antibody.

31. The method of any of claims 25-30, wherein binding of the IL13-Rα2 antibody to IL13-Rα2 is detected by flow cytometry, enzyme linked immunosorbent assay (ELISA), affinity chromatography, competitive inhibition assay, radioimmunoassay, immunofluorescence microscopy, immunoelectron microscopy, immunocytochemistry, or immunoprecipitation.

32. The method of any of claims 25-31, wherein the cell is a tumor cell.

33. The method of claim 32, wherein the cell is a malignant glioma cell.

34. The method of claim 32 or 33, wherein the cell is a glioblastoma cell.

35. A method for killing a cell that expresses an IL13-Rα2 comprising contacting the cell with an isolated antibody or fragment thereof that binds to the IL13-Rα2 and is conjugated to a cytotoxic agent, such that the IL13-Rα2 antibody binds the IL13-Rα2 and the cytotoxic agent contacts the cell, whereby the cell is killed.

36. The method of claim 35, wherein the antibody binds an epitope comprising an amino acid sequence of SEQ ID NO:1.

37. The method of claim 35, wherein the antibody binds an epitope consisting essentially of an amino acid sequence of SEQ ID NO:1.

38. The method of any of claims 35-37, wherein the antibody is a monoclonal antibody.

39. The method of any of claims 35-38, wherein the antibody is selected from the group consisting of a chicken antibody, a mouse antibody, a human antibody, and a humanized antibody.

40. The method of any of claims 35-39, wherein the antibody is a chicken IgY antibody.

41. The method of any of claims 35-40, wherein the cytotoxic agent is selected from the group consisting of a *Pseudomonas* exotoxin, a *Diphtheria* toxin, ricin, abrin, and a radionuclide.

42. The method of any of claims 35-41, wherein the cell is a tumor cell.

43. The method of claim 42, wherein the cell is a malignant glioma cell.

44. The method of claim 42 or 43, wherein the cell is a glioblastoma cell.

45. The method of any of claims 35-44, wherein the contacting comprises administering the IL13-Rα2 antibody to a human.

46. The method of claim 45, wherein the route of administration is intravenous, intraperitoneal, or intratumoral.

227836.ST25
SEQUENCE LISTING

<110> NeoPharm, Inc.

<120> IL 13 RECEPTOR ALPHA 2 ANTIBODY AND METHODS OF USE

<130> 227836

<150> US 60/457,898
<151> 2003-03-26

<160> 4

<170> PatentIn version 3.2

<210> 1
<211> 310
<212> PRT
<213> Artificial

<220>
<223> Synthetic

<400> 1

Ser Asp Thr Glu Ile Lys Val Asn Pro Pro Gln Asp Phe Glu Ile Val
1 5 10 15

Asp Pro Gly Tyr Leu Gly Tyr Leu Tyr Leu Gln Trp Gln Pro Pro Leu
20 25 30

Ser Leu Asp His Phe Lys Glu Cys Thr Val Glu Tyr Glu Leu Lys Tyr
35 40 45

Arg Asn Ile Gly Ser Glu Thr Trp Lys Thr Ile Ile Thr Lys Asn Leu
50 55 60

His Tyr Lys Asp Gly Phe Asp Leu Asn Lys Gly Ile Glu Ala Lys Ile
65 70 75 80

His Thr Leu Leu Pro Trp Gln Cys Thr Asn Gly Ser Glu Val Gln Ser
85 90 95

Ser Trp Ala Glu Thr Thr Tyr Trp Ile Ser Pro Gln Gly Ile Pro Glu
100 105 110

Thr Lys Val Gln Asp Met Asp Cys Val Tyr Tyr Asn Trp Gln Tyr Leu
115 120 125

Leu Cys Ser Trp Lys Pro Gly Ile Gly Val Leu Leu Asp Thr Asn Tyr
130 135 140

Asn Leu Phe Tyr Trp Tyr Glu Gly Leu Asp His Ala Leu Gln Cys Val
145 150 155 160

Asp Tyr Ile Lys Ala Asp Gly Gln Asn Ile Gly Cys Arg Phe Pro Tyr
165 170 175

Leu Glu Ala Ser Asp Tyr Lys Asp Phe Tyr Ile Cys Val Asn Gly Ser
180 185 190

Ser Glu Asn Lys Pro Ile Arg Ser Ser Tyr Phe Thr Phe Gln Leu Gln
195 200 205

Asn Ile Val Lys Pro Leu Pro Pro Val Tyr Leu Thr Phe Thr Arg Glu
210 215 220

Ser Ser Cys Glu Ile Lys Leu Lys Trp Ser Ile Pro Leu Gly Pro Ile
225 230 235 240

Pro Ala Arg Cys Phe Asp Tyr Glu Ile Glu Ile Arg Glu Asp Asp Thr
245 250 255

Thr Leu Val Thr Ala Thr Val Glu Asn Glu Thr Tyr Thr Leu Lys Thr
260 265 270

Thr Asn Glu Thr Arg Gln Leu Cys Phe Val Val Arg Ser Lys Val Asn
275 280 285

Ile Tyr Cys Ser Asp Asp Gly Ile Trp Ser Glu Trp Ser Asp Lys Gln
290 295 300

Cys Trp Glu Gly Glu Asp
305 310

<210> 2
<211> 930
<212> DNA
<213> Artificial

<220>
<223> Synthetic

<400> 2
tcagacaccg agataaaagt taaccctcct caggattttg agatagtgga tcccggatac 60

ttaggttatc tctatttgca atggcaaccc ccactgtctc tggatcattt taaggaatgc 120

acagtggaat atgaactaaa ataccgaaac attggtagtg aaacatggaa gaccatcatt 180

actaagaatc tacattacaa agatgggttt gatcttaaca agggcattga agcgaagata 240

cacacgcttt taccatggca atgcacaaat ggatcagaag ttcaaagttc ctgggcagaa 300

actacttatt ggatatcacc acaaggaatt ccagaaacta aagttcagga tatggattgc 360

gtatattaca attggcaata tttactctgt tcttggaaac ctggcatagg tgtacttctt 420

gataccaatt acaacttgtt ttactggtat gagggcttgg atcatgcatt acagtgtgtt 480

gattacatca aggctgatgg acaaaatata ggatgcagat ttccctattt ggaggcatca 540

gactataaag atttctatat ttgtgttaat ggatcatcag agaacaagcc tatcagatcc 600

agttatttca cttttcagct tcaaaatata gttaaacctt tgccgccagt ctatcttact 660

tttactcggg agagttcatg tgaaattaag ctgaaatgga gcataccttt gggacctatt 720

ccagcaaggt gttttgatta tgaaattgag atcagagaag atgatactac cttggtgact 780

gctacagttg aaaatgaaac atacaccttg aaaacaacaa atgaaacccg acaattatgc 840

tttgtagtaa gaagcaaagt gaatatttat tgctcagatg acggaatttg gagtgagtgg 900

agtgataaac aatgctggga aggtgaagac 930

<210> 3
<211> 1119
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Interleukin 13 receptor, alpha 2

<400> 3
atggctttcg tttgcttggc tatcggatgc ttatatacct ttctgataag cacaacattt 60

ggctgtactt catcttcaga caccgagata aaagttaacc ctcctcagga ttttgagata 120

gtggatcccg gatacttagg ttatctctat ttgcaatggc aacccccact gtctctggat 180

cattttaagg aatgcacagt ggaatatgaa ctaaaatacc gaaacattgg tagtgaaaca 240

tggaagacca tcattactaa gaatctacat tacaaagatg ggtttgatct taacaagggc 300

attgaagcga agatacacac gcttttacca tggcaatgca caaatggatc agaagttcaa 360

agttcctggg cagaaactac ttattggata tcaccacaag gaattccaga aactaaagtt 420

caggatatgg attgcgtata ttacaattgg caatatttac tctgttcttg gaaacctggc 480

ataggtgtac ttcttgatac caattacaac ttgttttact ggtatgaggg cttggatcat 540

gcattacagt gtgttgatta catcaaggct gatggacaaa atataggatg cagatttccc 600

tatttggagg catcagacta taaagatttc tatatttgtg ttaatggatc atcagagaac 660

aagcctatca gatccagtta tttcactttt cagcttcaaa atatagttaa acctttgccg 720

ccagtctatc ttacttttac tcgggagagt tcatgtgaaa ttaagctgaa atggagcata 780

cctttgggac ctattccagc aaggtgtttt gattatgaaa ttgagatcag agaagatgat 840

actaccttgg tgactgctac agttgaaaat gaaacataca ccttgaaaac aacaaatgaa 900

acccgacaat tatgctttgt agtaagaagc aaagtgaata tttattgctc agatgacgga 960

atttggagtg agtggagtga taaacaatgc tgggaaggtg aagacctatc gaagaaaact 1020

ttgctacgtt tctggctacc atttggtttc atcttaatat tagttatatt tgtaaccggt 1080

ctgcttttgc gtaagccaaa cacctaccca aaaatgatt 1119

<210> 4
<211> 380
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<223> Interleukin 13 receptor, alpha 2

<400> 4

Met Ala Phe Val Cys Leu Ala Ile Gly Cys Leu Tyr Thr Phe Leu Ile
1 5 10 15

Ser Thr Thr Phe Gly Cys Thr Ser Ser Ser Asp Thr Glu Ile Lys Val
20 25 30

Asn Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr
35 40 45

Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu
50 55 60

Cys Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr
65 70 75 80

Trp Lys Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp
85 90 95

Leu Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln
100 105 110

Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr
115 120 125

Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp
130 135 140

Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly
145 150 155 160

Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu
165 170 175

Gly Leu Asp His Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly
180 185 190

Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys
195 200 205

Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg
210 215 220

Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro
225 230 235 240

Pro Val Tyr Leu Thr Phe Thr Arg Glu Ser Ser Cys Glu Ile Lys Leu
245 250 255

Lys Trp Ser Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Asp Tyr
260 265 270

Glu Ile Glu Ile Arg Glu Asp Asp Thr Thr Leu Val Thr Ala Thr Val
275 280 285

Glu Asn Glu Thr Tyr Thr Leu Lys Thr Thr Asn Glu Thr Arg Gln Leu
290 295 300

Cys Phe Val Val Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly
305 310 315 320

Ile Trp Ser Glu Trp Ser Asp Lys Gln Cys Trp Glu Gly Glu Asp Leu
325 330 335

Ser Lys Lys Thr Leu Leu Arg Phe Trp Leu Pro Phe Gly Phe Ile Leu
340 345 350

Ile Leu Val Ile Phe Val Thr Gly Leu Leu Leu Arg Lys Pro Asn Thr
355 360 365

Tyr Pro Lys Met Ile Pro Glu Phe Phe Cys Asp Thr
370 375 380

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 October 2004 (14.10.2004)

PCT

(10) International Publication Number
WO 2004/087758 A3

(51) International Patent Classification[7]: C07K 16/28, A61K 47/48, A61P 35/00, A61K 51/10, G01N 33/68

(21) International Application Number: PCT/US2004/009354

(22) International Filing Date: 26 March 2004 (26.03.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/457,898 26 March 2003 (26.03.2003) US

(71) Applicant *(for all designated States except US)*: **NEOPHARM, INC.** [US/US]; 150 Field Drive, Suite 195, Lake Forest, IL 60045 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GATELY, Stephen, T.** [CA/US]; 357 E. Shady Pines Court, Palatine, IL 60067 (US). **WANASKI, Stephen, P.** [US/US]; 1301 West Fletcher, suite 503, Chicago, IL 60657 (US).

(74) Agents: **HEFNER, M., Daniel** et al.; Leydig, Voit & Mayer, Ltd., Two Prudential Plaza, Suite 4900, 180 North Stetson Av., Chicago, IL 60601-6780 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 20 January 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IL 13 RECEPTOR ALPHA 2 ANTIBODY AND METHODS OF USE

(57) Abstract: The invention provides an antiboy direted against an IL13 receptor alpha 2 (IL13-Rα2) and methods of using the antibody to detect an localize IL13-Rα2, to diagnostic a disease characterized by expression of IL13-Rα2, and to kill a cell that expresses an IL13-Rα2.

WO 2004/087758 A3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/009354

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/28 A61K47/48 A61P35/00 A61K51/10 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, PAJ, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/17968 A (US HEALTH ; PURI RAJ K (US)) 7 March 2002 (2002-03-07)	1-5, 7-15, 17-22, 24, 35-39, 41-46
	paragraph '0190!	
	paragraph '0033!	
Y	paragraph '0040!	1-46
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2004	29/10/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lechner, O

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/009354

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/58479 A (PENN STATE RES FOUND) 16 August 2001 (2001-08-16)	1-5,7, 10-15, 17-22, 24-29, 31-39, 42-45
Y	page 24, line 15 - page 26, line 20 page 11, lines 3-5,16-23 claims 27-32	1-46
Y	TINI M ET AL: "Generation and application of chicken egg-yolk antibodies" COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY PART A MOLECULAR AND INTEGRATIVE PHYSIOLOGY, vol. 131A, no. 3, March 2002 (2002-03), pages 569-574, XP002300522 ISSN: 1095-6433 abstract	1-46
A	OKANO FUMIYOSHI ET AL: "Identification of a novel HLA-A*0201-restricted, cytotoxic T lymphocyte epitope in a human glioma-associated antigen, interleukin 13 receptor alpha2 chain." CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH. SEP 2002, vol. 8, no. 9, September 2002 (2002-09), pages 2851-2855, XP002300523 ISSN: 1078-0432 abstract	1-46
A	BERNARD JEROME ET AL: "Expression of interleukin 13 receptor in glioma and renal cell carcinoma: IL13Ralpha2 as a decoy receptor for IL13" LABORATORY INVESTIGATION, vol. 81, no. 9, September 2001 (2001-09), pages 1223-1231, XP002300524 ISSN: 0023-6837 the whole document	1-46

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/009354

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 25-34 and 35-46 are directed to a diagnostic method practised on the human/animal body or method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2004/009354

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0217968 A	07-03-2002	AU 8497801 A	13-03-2002
		WO 0217968 A2	07-03-2002
WO 0158479 A	16-08-2001	AU 3689201 A	20-08-2001
		CA 2398136 A1	16-08-2001
		EP 1257289 A1	20-11-2002
		JP 2004511425 T	15-04-2004
		WO 0158479 A1	16-08-2001
		US 2002182219 A1	05-12-2002
		US 2002197266 A1	26-12-2002